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(52) Peptides with sulfate ester groups.

(54) Novel peptides having sulfate ester groups and containing 6 to 9 amino acids; possessing feeding inhibition properties and capable of stimulating the contraction of the gallbladder. Also methods of treating and preventing obesity in which these novel peptides or other specified peptides can be used.

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PEPTIDES WITH SULFATE ESTER GROUPSBACKGROUND OF THE INVENTION

This invention concerns sulfate ester containing peptides possessing feeding inhibition properties and capable of stimulating the contraction of the gallbladder. These peptides have 6 to 9 amino acids. They all differ structurally, however, from two similarly sized peptides known to have feeding inhibition properties: CCK-8, which has the structure, Asp-Tyr(SO₃H)-Met-Gly-Try-Met-Asp-Phe-NH₂, and ceruleotide, which has the structure, Glp-Gln-Asp-Tyr(SO₃H)-Thr-Gly-Trp-Met-Asp-Phe-NH₂. The peptides of this invention are not found in nature but, rather, must be synthesized.

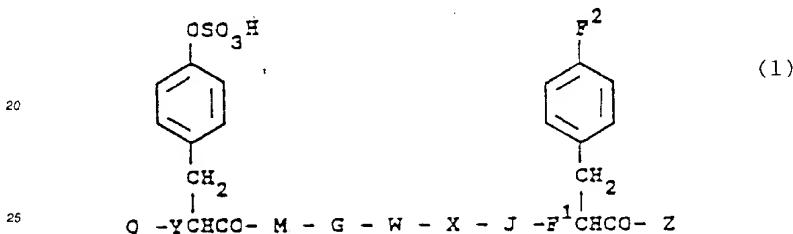
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SUMMARY OF THE INVENTION

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The compounds of the invention are peptides of the formula (1)

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wherein

Q is H, H-Asp, H- β Asp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R¹R²CHCOO,
 Y is H, (S)-NH, (R)-R³N, or (S)-R⁴N,
 20 M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, Melle, or Lys,
 G is Gly, DAla, Pro or Sar.
 W is Trp, MeTrp or Nal,
 X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, Pro, or Lys,
 J is Asp, DAsp, MeAsp, or Asn,
 35 F¹ is (S)-NH, (S)-R⁴N, or (R)-R⁴N,
 F² is H, Cl, I, Br, F, NO₂, NH₂, R⁵, or OR⁶,
 Z is NH₂, NHR⁷ or NR⁷R⁸,
 R¹ and R² are independently H or lower alkyl,
 R³, R⁴, and R⁵ are lower alkyl,
 40 R⁶ is H or lower alkyl, and
 R⁷ and R⁸ are lower alkyl,
 and pharmaceutically acceptable salts thereof,
 provided that
 (1) Q is desQ when Y is H,
 (2) F² is not H if, in the same peptide, Q is H-Asp or Ac, Y is (S)-NH, M is either Met, MetO, Ahx or Leu, X is either Met, MetO, Ahx or Leu, G is Gly, DAla or Pro, W is Trp, J is Asp, F¹ is (S)-NH, and Z is NH₂,
 (3) F² is not H if, in the same peptide, Q is H, H- β Asp or For, Y is (S)-NH, M is Met, Ahx or Leu, G is Gly, W is Trp, X is Met, Ahx or Leu, J is Asp, F¹ is (S)-NH, and Z is NH₂,
 (4) F² is not H if, in the same peptide, Y is H, M is Met, X is Met, G is Gly, W is Trp, J is Asp, F¹ is (S)-NH, and Z is NH₂, and
 (5) F² is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, G is Gly or DAla, W is Trp, J is Asp, F¹ is (S)-NH or (S)-R⁴N, and Z is NH₂.

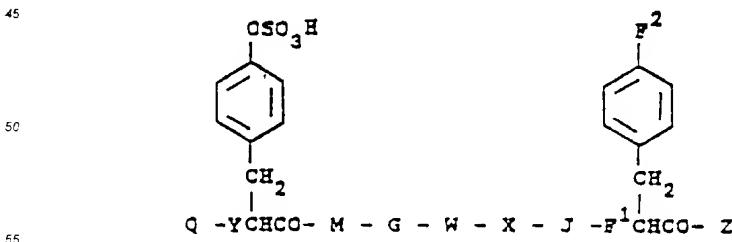
The invention is also a process of making the peptides of the invention.

The invention is also methods of treating obesity and preventing obesity, respectively; each such method comprising the administration, by either an intraperitoneal, intravenous, intramuscular, subcutaneous or intranasal route, to a mammal in need of such treatment, of a peptide of the formula (1) wherein

Q is H, H-Asp, H- β Asp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, H-Arg-Asp, Glp-Asp, Glp-Glu, Glp-Gln, Suc-
 5 Asp, Glt-Asp, Pht-Asp, R³CO-Asp, Boc-Asp, Cbz-Asp, H-Abu, H-Aia, Boc, Cbz, or R¹R²CHOCO,
 Y is H, (S)-NH, (R)-R³N, or (S)-R³N,
 M is Met, DMet, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Pro, Ile, Melle, Lys, Thr, Abu, Val, Mox,
 Gly, Phe, Tyr, or Trp,
 G is Gly, DAla, Sar, DTrp, Pro, or β Ala,
 10 W is Trp, MeTrp, Nal, DTrp, Trp(Me), Trp(5-F), or Trp(6-F),
 X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, Pro, Lys, DMet, Abu, or Mox,
 J is Asp, DAsp, MeAsp, β Asp, or Asn,
 F¹ is (S)-NH, (S)-R⁴N, (R)-NH, or (R)-R⁴N,
 F² is H, Cl, I, Br, F, NO₂, NH₂, R⁵, or OR⁶,
 15 Z is NH₂, NHR⁷ or NR⁷R⁸,
 R¹ and R² are independently H or lower alkyl,
 R³, R⁴ and R⁵ are lower alkyl,
 R⁶ is H or lower alkyl, and
 R⁷, R⁸ and R⁹ are lower alkyl,
 20 and pharmaceutically acceptable salts thereof,
 provided that
 (1) Q is desQ when Y is H, and
 (2) F² is not H if, in the same peptide, Q is H-Asp, Y is (S)-NH, M is Met, X is Met, G is Gly or Pro,
 W is Trp,
 25 J is Asp, F¹ is (S)-NH, and Z is NH₂.
 In its first subgeneric aspect, the invention is defined as compounds of the invention with the exception that it is further limited so that
 Q is H, H-Asp, H- β Asp, H-DAsp, For, Ac, Suc, desQ, or R¹R²CHOCO;
 Y is H, (S)-NH, or (S)-R³N;
 30 M is Met, MeMet, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, or Pro;
 G is Gly or DAla;
 W is Trp;
 X is Met, MeMet, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, or Pro;
 J is Asp;
 35 F¹ is (S)-NH or (S)-R⁴N;
 F² is H, Cl, NO₂, NH₂, R⁵ or OR⁶;
 and Z is NH₂.
 In a second subgeneric aspect, the invention is defined as in its first subgeneric aspect with the exception that it is further limited so that M is neither MeMet, MeAhx, MeLeu nor Melle, and X is neither
 40 MeMet, MeAhx, MeLeu, nor Melle.

Compounds with increased feeding inhibition activity over a 3-hour feeding period as compared to CCK-8 are:

A peptide of the formula



wherein

Q is H- β Asp, For, Suc, desQ, or R¹R²CHOCO,

Y is H or (S)-NH,
 M is Met, Ahx, Leu, or Ile,
 G is Gly,
 W is Trp,
 5 X is Met, Ahx, Leu, or Ile,
 J is Asp,
 F¹ is (S)-NH or (S)-R⁴N,
 F² is H, NO₂, R⁵, or OR⁶,
 Z is NH₂
 10 R¹ and R² are independently H or lower alkyl,
 R³, R⁴, R⁵, and R⁶ are lower alkyl,
 and pharmaceutically acceptable salts thereof,
 provided that
 (1) Q is desQ when Y is H,
 15 (2) F² is not H if, in the same peptide, Q is H-βAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or
 Leu, X is either Met, Ahx, or Leu, and F¹ is (S)-NH,
 (3) F² is not H if, in the same peptide, Y is H, M is Met, X is Met, and F¹ is (S)-NH,
 (4) F² is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F¹ is (S)-R⁴N.
 In additional subgeneric aspects of the invention, the method of treating obesity or the method of
 20 preventing obesity is further limited to the administration of either the compounds of the invention or the
 compounds identified in the first or second generic aspects of the invention.
 In a closely related invention, the compounds of the invention have Y as (R)-NH with the additional
 proviso that F² is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F¹ is (S)-NH,
 and Z is NH₂ so that compounds of this related invention are peptides of the formula (1) wherein
 25 Q is H, H-Asp, H-βAsp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R¹R²CHOCO.
 Y is (R)-NH,
 M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, Melle, or Lys,
 G is Gly, DAla, Pro or Sar,
 W is Trp, MeTrp or Nal,
 30 X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, Pro, or Lys,
 J is Asp, DAsp, MeAsp, or Asn,
 F¹ is (S)-NH, (S)-R⁴N, or (R)-R⁴N,
 F² is H, Cl, I, Br, F, NO₂, NH₂, R⁵, or OR⁶,
 Z is NH₂, NHR⁷, or NR⁷R⁸.
 35 R¹ and R² are independently H or lower alkyl,
 R³, R⁴, and R⁵ are lower alkyl,
 R⁶ is H or lower alkyl, and
 R⁷ and R⁸ are lower alkyl,
 and pharmaceutically acceptable salts thereof,
 40 provided that
 (1) F² is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F¹ is (S)-NH and Z is
 NH₂.

45 DETAILED DESCRIPTION

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Definitions

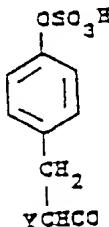
"lower alkyl" contains 1 to 6 carbon atoms.

(R) and (S) refer to the absolute configurations, about the adjacent methine carbon. When Y is (S)-NH, then

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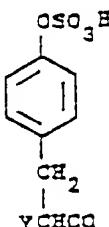


is of the L-configuration and when Y is (R)-NH, then

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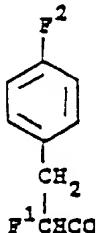
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is of the D-configuration. Similarly, when F¹ is (S)-NH or (R)-NH, then

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is of the L or D-configuration, respectively.

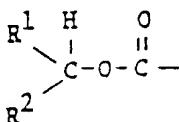
All optically active amino acids are of the L-configuration unless otherwise indicated.

The H in H-Asp, H- β Asp, H-DAsp, H-MeAsp, H-Arg, H-Abu, and H-Ala stands for hydrogen. DesQ, which arises when Y is H, means that there is no Q.

R¹R²CHCO is the formula

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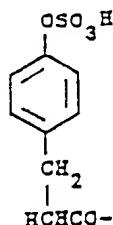
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Hpp(SO₃H) is the formula

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Each claim to a compound includes its pharmaceutically acceptable base addition salts. Base addition salts include those derived from both organic and inorganic bases, such as, for example, ammonia, sodium hydroxide, calcium hydroxide, barium hydroxide, tetraethylammonium hydroxide, ethylamine, diethylamine, triethylamine, and the like.

In an alternative representation to formula (1), used for purposes of brevity, peptides are also represented in accordance with conventional representation, for example, H-Asp-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂, which stands for the compound of formula (1) in which Q is H-Asp, Y is (R)-NH, M is Met, G is Gly, W is Trp, X is Met, J is Asp, F¹ is (S)-NH, F² is H, and Z is NH₂.

When amino acids, peptides, protecting groups, active groups, etc. are represented by symbols in this specification and appended claims, usual symbols as defined by IUPAC and IUB or as used in the art are employed. Examples of symbols are given below.

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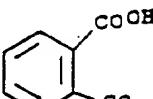
Abu.....	2-aminobutyric acid
Ac.....	acetyl
Ahx.....	2-aminohexanoic acid
Aib.....	2-aminoisobutyric acid
Ala.....	alanine
Arg.....	arginine
Asn.....	asparagine
Asp.....	aspartic acid
β Asp.....	beta-aspartic acid
Boc.....	tert-butyloxycarbonyl
BrCH ₂ -Pam.....	4-(bromomethyl)phenylacetamidomethyl
Cbz.....	carbobenzoxy
Cys(Me).....	S-methylcysteine
DAla.....	D-alanine
DAhx.....	D,2-aminohexanoic acid
DAsp.....	D-aspartic acid
DMet.....	D-methionine
DPhe.....	D-phenylalanine
DPhe-NH ₂	D-phenylalanine amide

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5	DTrp.....	D-tryptophan
	DTyr.....	D-tyrosine
	EtOCO.....	ethyloxycarbonyl
10	EtPhe.....	N-ethylphenylalanine
	EtPhe-NH ₂	N-ethylphenylalanine amide
15	Fmoc.....	9-fluorenylmethyloxycarbonyl
	For.....	formyl
	Gln.....	glutamine
20	Glp.....	pyroglutamyl
	Glu.....	glutamic acid
25	Glt.....	HOOC(CH ₂) ₃ CO-
	Gly.....	glycine
	His.....	histidine
30	Hpp.....	3-(4-hydroxyphenyl)propionyl
	Hpp(SO ₃ H).....	3-(O-sulfo-4-oxyphenyl)propionyl
35	iBuOCO.....	isobutyloxycarbonyl
	Ile.....	isoleucine
	Leu.....	leucine
40	Lys.....	lysine
	MeAhx.....	N-methyl-2-aminohexanoic acid
45	MeAsp.....	N-methylaspartic acid
	MeLeu.....	N-methylleucine
	MeIle.....	N-methylisoleucine
50	MeMet.....	N-methylmethionine
	MeOCO.....	methyloxycarbonyl
55	MePhe.....	N-methylphenylalanine

5	MePhe-NH ₂N-methylphenylalanine amide
	Met.....methionine
	MetO.....methionine sulfoxide
10	MeTrp.....N- α -methyltryptophan
	MeTyr.....N-methyltyrosine
15	MeTyr(Me).....N,O-dimethyltyrosine
	MeTyr(Me)-NH ₂N,O-dimethyltyrosine amide
	Mox.....methoxinine
20	Nal.....3-(2-naphthyl)alanine
	OBT.....1-benzotriazolyl ester
25	OCH ₂ -Pam.....4-(oxymethylphenyl)acetamidomethyl
	OSu.....succinimidylxyloxy ester
	OtBu.....tert-butyl ester
30	Phe.....phenylalanine
	Phe-NH ₂phenylalanine amide
35	Phe-NHET.....phenylalanine ethylamide
	Phe-NHMe.....phenylalanine methylamide
	Phe-N(Et) ₂phenylalanine diethylamide
40	Phe-N(Me) ₂phenylalanine dimethylamide
	Phe-OH.....phenylalanine acid
45	Phe(4-Cl).....3-(4-chlorophenyl)alanine
	Phe(4-Cl)-NH ₂3-(4-chlorophenyl)alanine amide
	Phe(4-Me).....3-(4-methylphenyl)alanine
50	Phe(4-Me)-NH ₂3-(4-methylphenyl)alanine amide
	Phe(4-NO ₂).....3-(4-nitrophenyl)alanine
	Phe(4-NO ₂)-NH ₂3-(4-nitrophenyl)alanine amide

	Phe(4-NH ₂).....	3-(4-aminophenyl)alanine
5	Phe(4-NH ₂)-NH ₂	3-(4-aminophenyl)alanine amide
10	Pht.....	
15	Pro.....	proline
20	Sar.....	sarcosine
25	Ser.....	serine
30	Suc.....	HOOC(CH ₂) ₂ CO-
35	tBu.....	tert-butyl
40	Thr.....	threonine
45	Trp.....	tryptophan
50	Trp(5-F).....	5-fluorotryptophan
	Trp(6-F).....	6-fluorotryptophan
	Trp(Me).....	1-methyltryptophan
	Tyr.....	tyrosine
	Tyr-NH ₂	tyrosine amide
	Tyr(Me).....	O-methyltyrosine
	Tyr(Me)-NH ₂	O-methyltyrosine amide
	Tyr(SO ₃ H).....	O-sulfotyrosine
	Tyr(SO ₃ H)-NH ₂	O-sulfotyrosine amide
	Val.....	valine

Preferred Compounds

The preferred compounds of the invention are those that during the 0.5 hour feeding period inhibited feeding by 48-100% when administered at 30μg/kg in the test described under "Utility" below. (See Table 2 below.)

The most preferred compound, at the time of filing this continuation-in-part application, from the point of view of gallbladder contraction, is the compound Hpp(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂.

Particularly preferred compounds, at the time of filing this continuation-in-part application, from the point of feeding inhibition, were those that during the 0.5 hour feeding period inhibited feeding by 20-72% when administered at 0.3 μ g/kg in the test described under "Utility" below. They are:

- H-DAsp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- 5 iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- H- β Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- H-DAsp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- For-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- 10 iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- Hpp(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- PrOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- EtOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- MeOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- 15 H- β Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Hpp(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- H-Asp-Tyr(SO₃H)-Leu-Gly-Trp-Leu-Asp-Phe-NH₂
- For-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- 20 Suc-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- 25 Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- H-DAsp-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- iBuOCO-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- 30 H-Asp-DTyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- Hpp(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂

The compound, EtOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂, was the one that inhibited feeding by 72% at 0.3 μ g/kg.

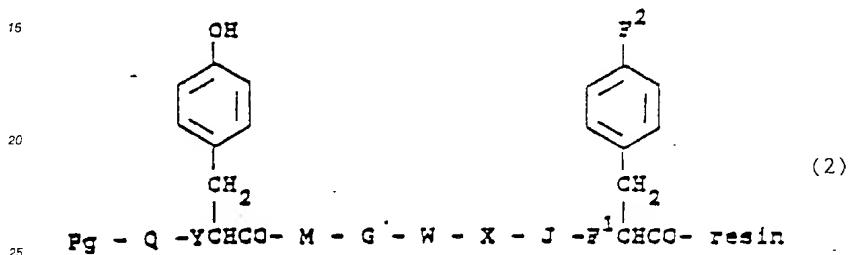
The most preferred compounds, at the time of filing this continuation-in-part application, from the point of view of feeding inhibition, were those that during the 3 hour feed period inhibited feeding by 50-90% when administered at 3 μ g/kg in the test described under "Utility" below. They are:

- iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- For-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- 40 iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- Hpp(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂
- PrOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- 45 iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂
- iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂
- Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-NH₂
- 50 For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- iBuOCO-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂
- Hpp(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂

The compound, Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, was the one that inhibited feeding by 90% at 3 μ g/kg during the 3 hour feeding period.

Preparation of Peptides

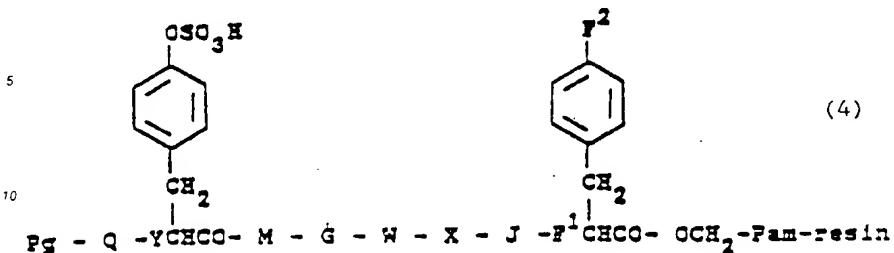
The novel sulfate ester peptides of this invention and the novel intermediates thereof may be prepared by methods well known to the art, for example, they may be prepared by combining individual amino acids on a solid phase resin on a step-by-step basis, or alternatively, by combining groups of amino acids on a solid phase resin to yield the desired peptidyl-resin intermediate. Such additions, as is known, are accomplished by protecting the amino group of the amino acid or group of amino acids by converting it to, for example, its tert-butyloxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc) derivative, and then activating the carboxylic group of such amino acid or group of amino acids by converting it, for example, to its 1-hydroxybenzotriazole (HOBt) or N-hydroxysuccinimide (HOsu) ester derivative. Such a protected-activated intermediate is then allowed to react with an amino acid-resin or peptidyl-resin with a free amino group, thus extending the peptide chain to provide the peptidyl-resin of formula 2, wherein Pg is a suitable protecting group, for example, Boc or Fmoc.



The phenolic OH group within formula (2) is converted to a sulfate ester by the use of a usual sulfating agent, such as sulfur trioxide pyridine complex. More specifically, the reaction is conducted, for example, by suspending a peptidyl-resin of formula 2 in dimethylformamide (DMF), pyridine or like solvent, and adding sulfur trioxide pyridine complex in about 10-40 molar excess to provide the sulfated peptidyl-resin of the formula 3.



45 Since the sulfate ester containing peptide end-products of this invention are C-terminal amides, the chemical link which connects the peptide chain to the resin must be such that its cleavage with suitable reagents readily provides amides. Due to the lability of the sulfate ester group to strong acids (for example, liquid hydrogen fluoride), the peptidyl-resin linkage may be cleavable with either weaker acids (for example, brief treatment with trifluoroacetic acid, TFA) and/or nucleophiles (for example, ammonia, amines, hydroxide, and alkoxides). Among the suitable resin derivatives may be mentioned oxymethyl-polystyrene, 4-
 50 (oxymethylphenyl)(CH₂)_nCO-aminomethyl-polystyrene ($n = 0-3$) and 4-(oxymethylphenyl)oxymethyl-polystyrene. Similarly substituted polyacrylamide resins are equally well suited as the above polystyrene based resins. For the purposes of this invention the 4-(oxymethylphenyl)CH₂CO-aminomethyl-polystyrene [herein referred to as 4-(oxymethylphenyl)acetamidomethylpolystyrene or OCH₂-Pam-resin] is best suited for the generation of peptide amides. Thus, this invention describes a process for the synthesis of sulfated peptidyl-OCH₂-Pam-resins of formula (4), wherein the resin is polystyrene (the term "polystyrene" includes copolymers with minor amounts, usually 1%, of unsaturated monomers such as divinylbenzene).

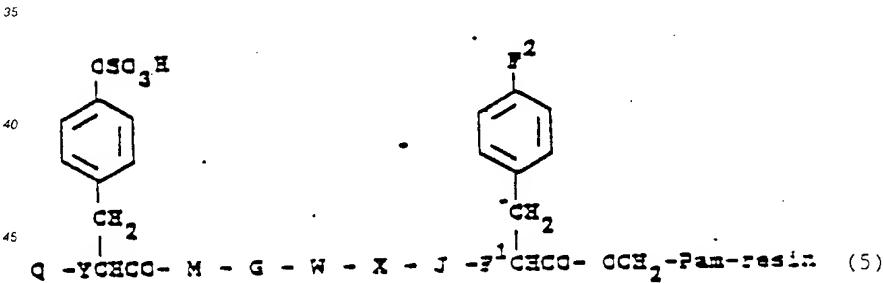


In forming peptide sequences of this invention, the amino functions may be protected by commonly used amino protecting groups such as Boc, Fmoc, (4-methoxybenzyl)oxycarbonyl, 2-nitrophenylsulfonyl, and so forth. The Boc and Fmoc protecting groups are preferred. The carboxyl and hydroxyl protecting group may be methyl, tert-butyl (tBu), benzyl, 4-methoxybenzyl and so forth. The tBu group is preferred.

The amino acid defined by the F¹ and F² groups of formula 4 may be attached to the OCH₂-Pam-resin in several ways. (a) For example, Boc protected-phenylalanine, wherein F¹ is (S)-NH and F² is H) may be reacted with a suitable 4-(bromomethyl)phenylacetate ester (for example, phenacyl ester) and processed further to provide Boc-Phe-(4-oxyethylphenyl)acetic acid which may be coupled to aminomethyl-polystyrene to provide Boc-Phe-(4-oxyethylphenyl)acetamidomethylpolystyrene (Boc-Phe-OCH₂-Pam-resin). (b) Alternatively, 4-(bromomethyl)phenylacetic acid may be coupled to aminomethylpolystyrene to provide 4-(bromomethyl)phenylacetamidomethylpolystyrene (BrCH₂-Pam-resin) which may be reacted with the cesium salt of Boc-Phe-OH to provide Boc-Phe-OCH₂-Pam-resin.

Among the suitable activating groups may be mentioned any combination of groups which causes the acid function of the amino acid to become more reactive, such as acid chlorides, mixed and symmetrical anhydrides, reaction product with carbodiimide (for example, dicyclohexylcarbodiimide, DCC), and active esters (for example, esters derived from HOBt, HOSu, 2- or 4-nitrophenol, and 2,4,5-trichlorophenol). The use of DCC and esters of HOBt and HOSu is particularly preferred from the standpoint of yield, lack of by-products, and consequent ease of purification.

The protecting groups are removed by known reactions such as treatment with dilute TFA (50% in dichloromethane, DCM) for Boc and/or tBu removal and treatment with dilute piperidine (20% in DMF) for Fmoc removal, to name a few, to provide the sulfated peptidyl-resin of the formula (5).



The sulfate ester containing peptides of formula 1 may be obtained by cleavage of the peptidyl-OCH₂-Pam-resin linkage of (5) with the appropriate reagent. The C-terminal sulfated peptide amides are derived, for example, by treatment of the sulfated peptidyl-resin of formula (5) with methanolic solutions of ammonia, alkylamines and dialkylamines.

An automatic peptide synthesizer was used for the solid phase synthesis of the sulfated peptide amides of this invention. The protocol of coupling onto aminomethyl-resin or peptidyl-OCH₂-Pam-resin (1 mmole of available nitrogen), deprotection, sulfation, cleavage, and product purification is set forth in Table 1.

Table 1. Protocol for solid phase synthesis of sulfated peptide amides (1 mmole scale). Each step volume is 50 ml unless otherwise indicated. All wash steps are repeated three times. Abbreviations: DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIET, N,N-diisopropylethylamine, DMF, dimethylformamide.

mamide; HOBT, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid.

Step	Reagent or Solvent	Purpose	Mix Time
1	DCM	Wash	1 min
2	Go to Step 3, 5, or 8	- - -	- - -
3	Add filtered, pre-activated (0°C, 1 hr) mixture of protected amino acid (or protected dipeptide, 3 mmole), HOBT (4.5 mmole), and DCC (3 mmole) in 1:4 DMF/DCM	Pre-activated DCC/HOBt coupling	2-15 hr
4	Go to Step 10, 16, 21, or 26	- - -	- - -
5	Add protected amino acid (or protected dipeptide, 3 mmole) and HOBT (4.5 mmole) in 30 ml 1:2 DMF/DCM then DCC (3 mmole) in 20 ml DCM	In situ activated DCC/HOBt coupling	2-15 hr
6	2-Propanol	Wash	1 min
7	Go to Step 4	- - -	- - -
8	Add active ester or anhydride (3 mmole) in DCM, DMF, or mixture thereof	Non DCC/HOBt activated coupling	2-15 hr
9	Go to Step 4	- - -	- - -
10	DCM	Wash	1 min
11	Treat with 49:1:50 TFA/anisole/DCM	Boc and tBu removal	30 min
12	DCM	Wash	1 min
13	Treat with 1:19 DIEA/DCM	Neutralize	1 min
14	DCM	Wash	1 min
15	Go to Step 1, 16, 21, or 26	- - -	- - -
16	DMF	Wash	1 min
17	Treat with 1:4 piperidine/DMF	Fmoc removal	3 min
18	Treat with 1:4 piperidine/DMF	Fmoc removal	7 min
19	DMF	Wash	1 min
20	Go to Step 15	- - -	- - -
21	DMF	Wash	1 min
22	1:2 pyridine/DMF	Wash	1 min
23	Add sulfur trioxide pyridine complex (40 mmole) in 60 ml 1:2 pyridine/DMF	Sulfation	20-24 hr
24	DMF	Wash	1 min
25	Go to Step 4	- - -	- - -
26	Methanol	Wash	1 min
27	Ammonia saturated (-20°C) methanol or 20% methanolic amine (250 ml)	Resin cleavage	2-5 day
28	Methanol	Wash	1 min
29	Combine, concentrate filtrates from Steps 27-28	Isolation	- - -
30	Chromatograph residue on column(s) of Amberlite XAD-2 (Rohm and Haas, 2.5 x 60 cm, methanol gradient 0.1 M in ammonia), Trisacryl M DEAE (LKB Inc., 2.5 x 47 cm, ammonium bicarbonate gradient), and/or P-40 ODS-3 (Whatman, 4.8 x 50 cm, methanol gradient 0.2% in ammonium acetate)	Purification	- - -

The sulfate ester containing peptides of formula (1) thus prepared may be desalted and purified by the usual methods. For example, the product may be purified by ion-exchange chromatography with use of Trisacryl M DEAE, DEAE-cellulose or the like, partition chromatography with use of Sephadex LH-20, Sephadex G-25 or the like, reverse phase chromatography with use of Amberlite XAD-2, ODS-silica gel or the like, normal phase chromatography with use of silica gel or the like, or high-performance liquid

chromatography (HPLC).

Analogous procedures, wherein the reactions are carried out without the solid phase component (resin), are well known in the art and well suited to large scale production. [See, e.g., U.S. Patent 3,892,726.]

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Utility

The peptides of this invention have the ability to inhibit feeding activity in mammals. As a result they have utility in the prevention and treatment of obesity. Feeding inhibition activity can be demonstrated in 10 rats as follows:

Male Sprague-Dawley rats (weighing 300-350 g) are individually caged and maintained on a 12 hr light, dark cycle and trained for at least 14 days to feed during a three hr period of the dark cycle but not the 21 hours preceding that three hr period. The day of the study, rats are dosed intraperitoneally with saline (controls) or test compound (dissolved in saline; usually at a concentration of 0.3 to 300 micrograms of test 15 compound per kg of rat weight). Food is introduced 10 minutes after administration of saline or test compound. Test compounds are deemed to be active if the test group consumes significantly less food than the saline controls during the feed period, which ends either 0.5 or 3 hr after presentation of food. The % feeding inhibition for the 1/2 hr and 3 hr feeding periods obtained by administering a dose of 30 µg/kg of test compound is given in Table 2 for CCK-8 and various compounds of the invention. For example, the % 20 feeding inhibition obtained with CCK-8 (first line of Table 2) was 70% for the 1/2 hr feeding period and 25% for the 3-hour period.

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TABLE 2

	<u>STRUCTURE</u>	% FEEDING INHIBITION $\frac{0.5 \text{ hr}}{3 \text{ hr}}$
10	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	70-25
	H-DAsp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	99-40
	iBuOCO-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	100-94
15	H-Asp-DTyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	74-24
	H-Asp-Tyr(SO ₃ H)-DMet-Gly-Trp-Met-Asp-Phe-NH ₂	17-IA
20	H-Asp-Tyr(SO ₃ H)-DAhx-Gly-Trp-Met-Asp-Phe-NH ₂	14-17*
	H-Asp-Tyr(SO ₃ H)-Met-Sar-Trp-Met-Asp-Phe-NH ₂	55*-12*
	H-Asp-Tyr(SO ₃ H)-Met-Gly-MeTrp-Met-Asp-Phe-NH ₂	31-11
25	H-Asp-Tyr(SO ₃ H)-Met-Gly-Nal-Met-Asp-Phe-NH ₂	52*-20*
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-DAsp-Phe-NH ₂	32-15*
30	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asn-Phe-NH ₂	43*-17*
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	70-24
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MeTyr(Me)-NH ₂	65-17
35	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe(4-NO ₂)-NH ₂	43-17
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe(4-Cl)-NH ₂	54-28*
40	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe(4-NH ₂)-NH ₂	27-IA
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Tyr(Me)-NH ₂	72*-10*
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NHMe	13*-IA
45	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NHEt	16-IA
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-N(Me) ₂	64-27
50	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-N(Et) ₂	18*-IA
	H-βAsp-DTyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	47-33*
	H-DAsp-DTyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	53-15
55	Suc-DTyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	82-60

5	H-Asp-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-Phe-NH ₂	85-20
	H-Asp-Tyr(SO ₃ H)-Lys-Gly-Trp-Lys-Asp-Phe-NH ₂	45*-IA
	Hpp(SO ₃ H)-Met-DAla-Trp-Met-Asp-Phe-NH ₂	59-16
10	Suc-Tyr(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH ₂	100-97
	H-βAsp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-78
15	H-DAsp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-72
	For-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-96
	iBuOCO-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-99
20	H-Asp-DTyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	85-35
	Hpp(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-100
25	Hpp(SO ₃ H)-Met-DAla-Trp-Met-Asp-MePhe-NH ₂	69-27
	PrOCO-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	90-74
	EtOCO-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	89-63
30	MeOCO-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	100-91
	H-βAsp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	91-21
35	H-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	50-30*
	Suc-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	75-55
	Hpp(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	99-75
40	H-Asp-Tyr(SO ₃ H)-Ahx-Gly-Trp-Met-Asp-Phe-NH ₂	NT-NT
	H-Asp-Tyr(SO ₃ H)-Met-Gly-DTrp-Met-Asp-Phe-NH ₂	42*-IA
45	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Ahx-Asp-Phe-NH ₂	82-23
	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-DPhe-NH ₂	46-14*
	H-Asp-Tyr(SO ₃ H)-MetO-Gly-Trp-MetO-Asp-Phe-NH ₂	15-IA
50	H-Asp-Tyr(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH ₂	82-27
	H-Asp-Tyr(SO ₃ H)-Leu-Gly-Trp-Leu-Asp-Phe-NH ₂	89-24
55	For-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	100-66

5	H-Asp-Tyr(SO ₃ H)-MetO-Gly-Trp-Met-Asp-Phe-NH ₂	89-25
10	H-Asp-Tyr(SO ₃ H)-Met-DAla-Trp-Met-Asp-Phe-NH ₂	16-18*
15	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-DMet-Asp-Phe-NH ₂	30*-IA
20	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-MetO-Asp-Phe-NH ₂	45-21*
25	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe(4-Me)-NH ₂	57-18
30	H-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Tyr-NH ₂	23*-IA
35	Hpp(SO ₃ H)-Met-Gly-Trp-Pro-Asp-Phe-NH ₂	48-IA
40	Hpp(SO ₃ H)-Pro-Gly-Trp-Pro-Asp-Phe-NH ₂	25-IA
45	Suc-Tyr(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH ₂	100-98
50	iBuOCO-Tyr(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH ₂	100-100
55	Hpp(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH ₂	94-96
60	iBuOCO-Tyr(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH ₂	100-100
65	Hpp(SO ₃ H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH ₂	100-99
70	Suc-Tyr(SO ₃ H)-Met-DAla-Trp-Met-Asp-Phe-NH ₂	75-30
75	For-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-Phe-NH ₂	NT-NT
80	Suc-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-Phe-NH ₂	73-54
85	iBuOCO-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-Phe-NH ₂	62-58
90	Hpp(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-Phe-NH ₂	87-85
95	Suc-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-MePhe-NH ₂	100-95
100	H-DAsp-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	95-75
105	For-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	100-85
110	Suc-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	100-100
115	iBuOCO-Tyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	96-92
120	H-Asp-DTyr(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	40-IA
125	Hpp(SO ₃ H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH ₂	100-97

IA means "inactive".

* - <10% inhibition at 30 µg/kg; number shown represents %
inhibition at dose of 300 µg/kg.

NT means "not tested"

(END OF TABLE 2)

An appropriate procedure for administering a peptide designated here as suitable for intraperitoneal, intravenous, intramuscular, subcutaneous, or intranasal administration, to a mammal in need of either treatment for obesity or prevention of obesity, is at a dose of about 0.3 micrograms (µg) to 3 mg per kg of body weight per day, either as single dose or divided among two to four doses. The dosage may be varied, depending upon the requirements of the patient and the compound being employed.

The peptides of this invention have the ability to stimulate gallbladder contraction in mammals. Thus, they also find utility as diagnostic aids in X-ray examination of the gallbladder. The use of gallbladder contracting agents as diagnostic aids is a well established medical procedure.

EXAMPLES

The invention may be further illustrated by the following examples. Examples 1-18 illustrate the synthesis of intermediates. Examples 19-93 illustrate the synthesis of compounds that have been designated above as either a compound of the invention or a compound useful in the treatment and prevention of obesity. The examples are intended to illustrate the invention, not to limit it in any manner.

Peptide syntheses, unless otherwise stated, were initiated with 1 milliequivalent of aminomethyl resin, where the resin was 99:1 by weight styrene:divinylbenzene copolymer. Reactions were performed at room temperature unless otherwise stated.

Washing steps were performed three times with 50 ml of the specified solvent unless otherwise stated.

EXAMPLE 1

Isobutyl Succinimidyl Carbonate (iBuOCO-OSu)

To a solution of isobutyl chloroformate (26 ml, 200 mmole) in 600 ml of chloroform was added in portions the dicyclohexylamine (DCHA) salt of N-hydroxysuccinimide (HOSu, 49.28 g, 200 mmole). After stirring the resulting suspension overnight, the precipitated DCHA hydrochloride was filtered off and washed with chloroform. The concentrated filtrate (ca. 50 ml) and washings were diluted with 400 ml of ethyl acetate (EtOAc) and washed with 10% citric acid (4 x 100 ml), brine (2 x 100 ml), 10% sodium bicarbonate (3 x 100 ml), and brine (4 x 100 ml) and then dried (magnesium sulfate), filtered, and concentrated to ca. 100 ml. On diluting with ether and precipitating with hexane, 26.6 g (62% yield) of iBuOCO-OSu was obtained, mp 33-35°C.

EXAMPLE 2

Fmoc-DTyr-OH

D-Tyrosine (1.81 g) was dissolved in 20 ml of water and 30 ml of tetrahydrofuran (THF) with 10 ml of N sodium hydroxide. Solid 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 3.37 g) was added with rapid stirring. The suspension was adjusted to pH 7 with N sodium hydroxide and stirred overnight. Solid citric acid (3 g) was added followed by 60 ml of EtOAc. The EtOAc layer was collected, washed with 10% citric acid, brine, and dried (magnesium sulfate). Evaporation of the EtOAc solution gave a light tan syrup which was crystallized from dichloromethane (DCM) to give 3.9 g of Fmoc-DTyr-OH, mp 178-181°C.

EXAMPLE 3

Fmoc-Tyr-OH

5 Following the procedure of Example 2 but substituting L-tyrosine (9.06 g) for D-tyrosine, 18.4 g of Fmoc-Tyr-OH was obtained, mp 172-177°C.

EXAMPLE 4

10 Fmoc-MeTyr-OH

Following the procedure of Example 2 but substituting N-methyltyrosine (1.95 g) for D-tyrosine, 1.22 g of Fmoc-MeTyr-OH was obtained, mp 152-158°C.

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EXAMPLE 5

Boc-MePhe-(4-oxymethylphenyl)acetic Acid

20 To a solution of Boc-MePhe-OH (27.93 g) and 4-(bromomethyl) phenylacetic acid phenacyl ester (33.32 g) in 1000 ml of acetonitrile was added potassium fluoride dihydrate (18.28 g). The suspension was stirred overnight, filtered and the filtrate evaporated to dryness. The residue, Boc-MePhe-(4-oxymethylphenyl)-acetic acid phenacyl ester, was dissolved in 85% acetic acid (1200 ml), treated with zinc dust (128 g), and 25 stirred for 2-4 hrs. Concentration of the filtered reaction mixture to ca. 400 ml and dilution with ca. 3200 ml of water gave an oil which was dissolved in EtOAc and treated with DCHA to give 41.31 g of the DCHA salt of title compound, mp 120-122°C.

30 EXAMPLE 6

Boc-EtPhe-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Boc-EtPhe-OH (7.33 g) for Boc-MePhe-OH, 5.69 g of the DCHA salt of Boc-EtPhe-(4-oxymethylphenyl)acetic acid was obtained, mp 137-141°C.

EXAMPLE 7

40 Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Boc-Phe(4-Cl)-OH (2.5 g) for Boc-MePhe-OH, 3.44 g of the free base of Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic acid was obtained.

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EXAMPLE 8

Boc-Tyr(Me)-(4-oxymethylphenyl)acetic Acid

50 Following the procedure of Example 5 but substituting Boc-Tyr(Me)-OH (2.5 g) for Boc-MePhe-OH, 1.83 g of the free base of Boc-Tyr(Me)-(4-oxymethylphenyl)acetic acid was obtained, mp 64-67°C

55 EXAMPLE 9

Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic Acid

Following the procedure of Example 5 but substituting Fmoc-Tyr(tBu)-OH (6.86 g) for Boc-MePhe-OH, 4.88 g of the free base of Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic acid was obtained, mp 192-195°C.

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EXAMPLE 10

Fmoc-Met-Asp(OtBu)-OH

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Fmoc-Met-Osu was prepared, *in situ*, by the reaction of Fmoc-Met-OH (14.87 g), HOSu (5.52 g), and dicyclohexylcarbodiimide (DCC, 8.26 g) in THF (200 ml) at 0°C for 3.5 hrs. Precipitated dicyclohexylurea (DCU) was removed by filtration and the THF filtrate was added to a cold solution of H-Asp (OtBu)-OH in 220 ml of 10:1 water/THF to which had been added 40 ml of N sodium hydroxide. After stirring the reaction mixture at room temperature overnight, solid citric acid (20 g) was added along with EtOAc (600 ml). The EtOAc layer was separated, washed with 10% citric acid and brine, and dried (magnesium sulfate). Evaporation of the EtOAc solution gave a residue which was dissolved in 200 ml of EtOAc and treated with DCHA (7.84 ml) to precipitate 17.93 g of the DCHA salt of the desired product, mp 159-162°C.

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EXAMPLE 11

H-Phe-OCH₂-Pam-resin

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Boc-Phe-(4-oxymethylphenyl)acetic acid (0.83 g, 2 mmole), 1-hydroxybenzotriazole (HOBT, 0.46 g, 3 mmole) and DCC (0.41 g, 2 mmole) were dissolved in 50 ml of 4:1 DCM/DMF and stirred at 0°C for 1 hr. Aminomethyl-resin (1.34 g, 1 mmole available nitrogen (was suspended in the filtered reaction mixture (precipitated DCU removed) and shaken for 2 to 15 hours. The product, Boc-Phe-OCH₂-Pam-resin, was isolated by filtration and treated according to Table 1 (Steps 10-14) to give the desired free base, H-Phe-OCH₂-Pam-resin.

Example 12

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H-MePhe-OCH₂-Pam-resin

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Boc-MePhe-(4-oxymethylphenyl)acetic acid (from 1.82 g, 3 mmole, of its DCHA salt, Example 5) and HOBT (0.69 g, 4.5 mmole) in 40 ml of 1:3 DMF/DCM followed by DCC (0.62 g, 3 mmole) in 20 ml of DCM were added to aminomethyl-resin (1.34 g, 1 mmole available nitrogen) to give a suspension which was shaken for 2 to 15 hours. The desired product, Boc-MePhe-OCH₂-Pam-resin, was isolated by filtration, washed with 2-propanol and DCM, and treated according to Table 1 (Steps 10-14) to give the desired free base, H-MePhe-OCH₂-Pam-resin.

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EXAMPLE 13

H-EtPhe-OCH₂-Pam-resin

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Following the procedure of Example 12 but substituting Boc-EtPhe-(4-oxymethylphenyl)acetic acid (from 1.87 g, 3 mmole, of its DCHA salt, Example 6) for Boc-MePhe-(4-oxymethylphenyl)acetic acid, H-EtPhe-OCH₂-Pam-resin was obtained.

EXAMPLE 14

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H-Phe(4-Cl)-OCH₂-Pam-resin

Following the procedure of Example 11 but substituting Boc-Phe(4-Cl)-(4-oxymethylphenyl)acetic acid (0.90 g, 2 mmole, Example 7) for Boc-Phe-(4-oxymethylphenyl)acetic acid, H-Phe(4-Cl)-OCH₂-Pam-resin was obtained.

EXAMPLE 15

10 H-Phe(4-NO₂)-OCH₂-Pam-resin

Boc-Phe(4-NO₂)-OH (1.39 g) was dissolved in 100 ml of 70% methanol (MeOH) and adjusted to pH 7 with the addition of N cesium bicarbonate. The solution was evaporated to dryness with the residue being evaporated three more times with added DMF. The resultant dried cesium salt of Boc-Phe(4-NO₂)-OH was dissolved in 60 ml of DMF and shaken with BrCH₂-Pam-resin (1 meq or Br) overnight. The desired product, Boc-Phe(4-NO₂)-OCH₂-Pam-resin, was isolated by filtration, washed with DCM, and treated according to Table 1 (Steps 10-14) to give the desired free base, H-Phe(4-NO₂)-OCH₂-Pam-resin.

20 EXAMPLE 16

H-Tyr(Me)-OCH₂-Pam-resin

Following the procedure of Example 11 but substituting Boc-Tyr(Me)-(4-oxymethylphenyl)acetic acid (0.87 g, 2 mmole, Example 8) for Boc-Phe-(4-oxymethylphenyl)acetic acid, H-Tyr(Me)-OCH₂-Pam-resin was obtained.

EXAMPLE 17

30 H-Tyr(tBu)-OCH₂-Pam-resin

Fmoc-Tyr(tBu)-(4-oxymethylphenyl)acetic acid (1.82 g, 3 mmole, Example 9), 1-hydroxybenzotriazole (HOBr, 0.69 g, 4.5 mmole), and DCC (0.62 g, 3 mmole) were dissolved in 50 ml of 4:1 DCM/DMF and stirred at 0°C for 1 hr. Aminomethylresin (1.34 g, 1 mmole available nitrogen) was suspended in the filtered reaction mixture (precipitated DCU removed) and shaken for 2 to 15 hours. The product, Fmoc-Tyr(tBu)-OCH₂-Pam-resin, was isolated by filtration and treated according to Table 1 (Steps 16-20) to give the desired free base, H-Tyr(tBu)-OCH₂-Pam-resin.

40 EXAMPLE 18

H-MeTyr(Me)-OCH₂-Pam-resin

45 Following the procedure of Example 15 but substituting Boc-MeTyr(Me)-OH (from 1.47 g of its DCHA salt) for Boc-Phe(N₂)-OH, H-MeTyr(Me)-OCH₂-Pam-resin was obtained.

EXAMPLE 19

50 H-DAsp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-DAsp(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step

30) to give 198 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2), Tyr 1.04 (1), Met 2.07 (2), Gly 1.08 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35 (TLC herein refers to chromatography of the title compound on Merck silica gel thin layer plates in the solvent system chloroform-methanol-acetic acid-water, 6:3:1:1.

5 EXAMPLE 20

10 iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 206 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.06 (1), Tyr 1.04 (1), Met 2.04 (2), Gly 1.06 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.56.

25 EXAMPLE 21

H-Asp-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr-OH (Example 2), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Boc-Asp(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 241 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.14 (2), Tyr 1.06 (1), Met 2.12 (2), Gly 0.95 (1), Phe 0.98 (1), and NH₃ 1.30 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.32.

40 EXAMPLE 22

H-Asp-Tyr(SO₃H)-DMet-Gly-Trp-Met-Asp-Phe-NH₂

45 H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-DMet-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-DMet-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 219 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.07 (2), Tyr 1.02 (1), Met 2.11 (2), Gly 0.95 (1), Phe 1.11 (1), and NH₃ 1.31 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

55

EXAMPLE 23

H-Asp-Tyr(SO₃H)-DAhx-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-DAhx-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-DAhx-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 248 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2), Tyr 1.03 (1), Ahx 0.97 (1), Gly 0.99 (1), Met 1.07 (1), and Phe 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.34.

15 EXAMPLE 24

H-Asp-Tyr(SO₃H)-Met-Sar-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Sar-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Sar-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.93 (2), Tyr 0.97 (1), Met 2.00 (2), Sar 1.03 (1), Phe 1.02 (1), and NH₃ 1.54 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.32.

30 EXAMPLE 25

H-Asp-Tyr(SO₃H)-Met-Gly-MeTrp-Met-Asp-Phe-NH₂

35 H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-MeTrp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-MeTrp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 310 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 1.02 (1), Met 1.95 (2), Gly 0.93 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35.

45 EXAMPLE 26

H-Asp-Tyr(SO₃H)-Met-Gly-Nal-Met-Asp-Phe-NH₂

50 H-Phe-OCH₂-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Nal-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Nal-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step

30) to give 260 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.91 (1), Met 2.13 (2), Gly 1.09 (1), Nal 0.76 (1), and Phe 1.14 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.38.

5

EXAMPLE 27

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-DAsp-Phe-NH₂

10

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp-(OtBu)-Tyr-Met-Gly-Trp-Met-DAsp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 121 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 0.98 (1), Met 2.05 (2), Gly 1.07 (1), and Phe 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.43.

20

EXAMPLE 28

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asn-Phe-NH₂

25

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asn-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asn-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.03 (1), Met 1.93 (2), Gly 1.01 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.43.

EXAMPLE 29

40 H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (free base of Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp-(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 243 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.14 (2), Tyr 1.02 (1), Met 2.04 (2), Gly 1.07 (1), and NH₃ 1.87 (2). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

55

EXAMPLE 30

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MeTyr(Me)-N₂

H-MeTyr(Me)-OCH₂-Pam-resin (Example 18) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (free base of Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MeTyr(Me)-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.06 (2), Tyr 1.06 (1), Met 1.98 (2), and Gly 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.45.

15 EXAMPLE 31

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe(4-NO₂)-NH₂

H-Phe(4-NO₂)-OCH₂-Pam-resin (Example 15) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-NO₂)-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 128 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.98 (2), Tyr 1.05 (1), Met 1.92 (2), and Gly 1.05(1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

30 EXAMPLE 32

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe(4-Cl)-NH₂

H-Phe(4-Cl)-OCH₂-Pam-resin (Example 14) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-Cl)-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 299 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.85 (2), Tyr 1.02 (1), Met 1.78 (2), and Gly 0.92 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.36.

45 EXAMPLE 33

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe(4-NH₂)-NH₂

50 H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe(4-NO₂)-NH₂ (64 mg, Example 31) was dissolved in 85% acetic acid (20 ml) and treated with zinc dust (69 mg) with stirring. After 30 min, the filtered reaction mixture was evaporated to dryness and the residue was chromatographically purified on P-40 ODS-3 according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.92 (2), Tyr 0.98 (1), Met 1.87 (2), and Gly 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35.

EXAMPLE 34

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Tyr(Me)-NH₂

5 H-Tyr(Me)-OCH₂-Pam-resin (Example 16) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Tyr(Me)-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 10-15 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 313 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.05 (2), Tyr 1.98 (2), Met 1.88 (2), and Gly 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.38.

15

EXAMPLE 35

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NHMe

20 H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and 25 cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with methylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 196 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.99 (2), Tyr 1.00 (1), Met 1.98 (2) Gly 1.00 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong 30 peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.47.

EXAMPLE 36

35 H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NHEt

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ethylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 180 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.99 (2), Tyr 0.84 (1), Met 2.03 (2), Gly 1.01 (1), Phe 0.97 (1), and NH3 1.14 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.49.

EXAMPLE 37

50 H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-N(Me)₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with dimethylamine) to give the title compound which was chromatographically purified on Trisacryl M

DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.01 (1), Met 1.97 (2), Gly 0.99 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35.

5

EXAMPLE 38

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-N(Et)₂

10

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with diethylamine) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 102 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.16 (2), Tyr 1.03 (1), Met 1.92 (2), Gly 1.00 (1), and Phe 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.38.

20

EXAMPLE 39

25 H-βAsp-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr-OH (Example 2) and Boc-βAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Boc-βAsp(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-15) and coupled with Fmoc-OSu (1.1 g) according to Table 1 (Steps 8-9) to give Fmoc-βAsp-DTyr-Met-Gly-Trp-Met-Asp-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 121 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.94 (2), Tyr 1.02 (1), Met 1.91 (2), Gly 1.11 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

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EXAMPLE 40

H-DAsp-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

45 H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr-OH (Example 2) and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-DAsp-(OtBu)-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.08 (2), Tyr 1.06 (1), Met 1.83 (2), Gly 1.05 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.24.

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EXAMPLE 41

Suc-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-DTyr-OH (Example 2) according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give H-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with succinic anhydride (0.6 g, 6 mmole, in DMF) according to Table 1 (Steps 8-9) to give Suc-DTyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 290 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.08 (2), Tyr 1.04 (1), Met 1.70 (2), Gly 1.14 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.51.

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EXAMPLE 42

H-Asp-DTyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ile-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp(OtBu)-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 370 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.05 (2), Tyr 1.00 (1), Ile 1.95 (2), Gly 1.00 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.33.

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EXAMPLE 43

H-Asp-Tyr(SO₃H)-Lys-Gly-Trp-Lys-Asp-Phe-NH₂

25

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Lys-(Boc)-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp(OtBu)-Tyr(tBu)-Lys(Boc)-Gly-Trp-Lys(Boc)-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 61 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.98 (2), Tyr 1.01 (1), Lys 2.00 (2) Gly 0.99 (1), and Phe 1.02 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.10.

EXAMPLE 44

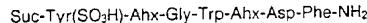
Hpp(SO₃H)-Met-DAla-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-DAla-OH, and Fmoc-Met-OH, according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with Hpp-OSu according to Table 1 (Steps 8-9) to give Hpp-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatog-

raphically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 140 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02(1), Met 1.97 (2), Ala 0.98 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.52.

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EXAMPLE 45



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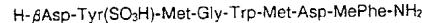
H-Phe-OCH₂-Pam resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH, according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with succinic anhydride (0.6 g, 6 mmole, in DMF) according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.01 (1), Tyr 0.95 (1), Ahx 2.10 (2), Gly 1.06 (1), and Phe 0.88 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.36.

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EXAMPLE 46

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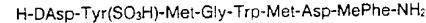
H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Boc-βAsp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give Boc-βAsp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-15) and coupled with Fmoc-OSu 1.1 g) according to Table 1 (Steps 8-9) to give Fmoc-βAsp-Tyr-Met-Gly-Trp-Met-Asp-MePhe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 72 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 1.05 (1), Met 1.85 (2), and Gly 1.10 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.33.

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EXAMPLE 47

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H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-DAsp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 110 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.01 (2), Tyr 1.01 (1), Met 1.91 (2), and Gly 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35.

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EXAMPLE 48

For-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give Fmoc-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-20) and coupled with For-Tyr-OH according to Table 1 (Steps 5-7) to give For-Tyr-Met-Gly-Trp-Met-Asp-MePhe-OCH₂-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 78 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Tyr 1.01 (1), Met 1.95 (2), and Gly 1.02 (1). Infrared absorption spectrum showed a strom peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.45.

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EXAMPLE 49

iBuOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 302 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.03 (1), Tyr 1.02 (1), Met 1.91 (2), and Gly 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.67.

EXAMPLE 50

35 H-Asp-DTyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-DTyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give Fmoc-Asp(OtBu)-DTyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 29 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 0.97 (1), Met 1.93 (2), and Gly 1.07 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.28.

EXAM 51

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Hpp(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to give H-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with 3-(4-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester (Hpp-OSu) according to Table 1 (Steps 8-9) to give Hpp-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then

Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 170 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Met 1.97 (2), and Gly 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.58.

EXAMPLE 52

10 Hpp(SO₃H)-Met-DAla-Trp-Met-Asp-MePhe-NH₂

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-DAla-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Met-DAla-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin 15 which was coupled with Hpp-OSu according to Table 1 (Steps 8-9) to give Hpp-Met-DAla-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 84 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 0.99 (1), Met 1.94 (2), and Ala 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.49.

EXAMPLE 53

25 PrOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling 30 Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with PrOCO-OSu (mp 31-34°C, prepared according to the procedure of Example 1 except that propyl chloroformate was substituted for isobutyl chloroformate) according to Table 1 (Steps 8-9) to give PrOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 35 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 270 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.01 (1), Tyr 1.03 (1), Met 1.87 (2), Gly 1.02 (1), and Phe 1.06 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.45.

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EXAMPLE 54

45 EtOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Tyr-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with EtOCO-OSu (mp 52-54.5°C, prepared according to the procedure of Example 1 except that ethyl chloroformate was substituted for isobutyl chloroformate) according to Table 1 (Steps 8-9) to give EtOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 50 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 300 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.02 (1), Tyr 1.01 (1), Met 1.91 (2), Gly 1.02 (1), and Phe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.44.

EXAMPLE 55

MeOCO-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

5 H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-
 OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling
 Steps 5-7 followed by Fmoc removal Steps 16-20) to give H-Typ-(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-
 OCH₂-Pam-resin which was coupled with MeOCO-OSu (mp 87-89°C, prepared according to the procedure
 of Example 1 except that methyl chloroformate was substituted for isobutyl chloroformate) according to
 10 Table 1 (Steps 8-9) to give MeOCO-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was
 deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then
 15 Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite
 XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 270 mg of the ammonium salt
 of the title compound. Amino acid analysis following acid decomposition gave Asp 1.04 (1), Tyr 1.05 (1),
 Met 1.82 (2), Gly 1.03 (1), and Phe 1.06 (1). Infrared absorption spectrum showed a strong peak typical of a
 sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.44.

EXAMPLE 56

20 H-βAsp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

The title compound has previously been prepared (Digestive Diseases, 15, 149-156 (1970)). H-Phe-
 OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-
 25 Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-βAsp(OtBu)-OH according to
 Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-βAsp(OtBu)-Tyr-Met-
 Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin
 according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title
 30 compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to
 give 283 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition
 gave Asp 2.03 (2), Tyr 0.94 (1), Met 2.08 (2), Gly 0.99 (1), and Phe 0.96 (1). Infrared absorption spectrum
 showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.52.

35 EXAMPLE 57

H-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

The title compound has previously been prepared (U.S. Patents 3,839,315 and 3,705,140). H-Phe-OCH₂-
 40 Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH,
 Fmoc-Gly-OH, Fmoc-Met-OH, and Boc-Tyr-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc
 removal Steps 16-20) to provide Boc-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sul-
 fated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then
 45 Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl
 M DEAE according to Table 1 (Step 30) to give 240 mg of the ammonium salt of the title compound. Amino
 acid analysis following acid decomposition gave Asp 1.05 (1), Tyr 1.04 (1), Met 2.10 (2), Gly 1.07 (1), and
 Phe 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050
 cm⁻¹. TLC R_f 0.55.

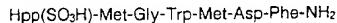
50 EXAMPLE 58

Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

55 The title compound has previously been prepared (European Patent Application 0107860). H-Phe-OCH₂-
 Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH,
 Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr-OH (Example 3) according to Table 1 (coupling Steps 3-4
 followed by Fmoc removal Steps 16-20) to provide H-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin

which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 246 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.03 (1), Tyr 0.95 (1), Met 2.08 (2), Gly 0.98 (1), and Phe 0.96 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.54.

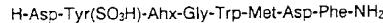
10 EXAMPLE 59



The title compound has previously been prepared (Int. J. Peptide Protein Res., 16, 402-411 (1980)). H-15 Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 65 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.04 (1), Met 2.04 (2), Gly 0.95 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.47.

25

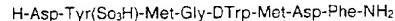
EXAMPLE 60



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The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Ahx-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 188 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.99 (1), Ahx 1.06 (1), Gly 1.07 (1), Met 0.93 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.57.

45 EXAMPLE 61



The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-DTrp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-DTrp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 314 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.07 (2), Tyr 1.01 (1), Met 2.01 (2), Gly 1.06 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.30.

EXAMPLE 62



5 The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH₂-Pam-resin
 (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-
 OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling
 Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp (OtBu)-Tyr-Met-Gly-Trp-Ahx-Asp-
 (OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to
 10 Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which
 was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 87 mg of the
 ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.11 (2),
 Tyr 1.00 (1), Met 1.11 (1), Gly 1.02 (1), Ahx 1.04 (1), and Phe 0.99 (1). Infrared absorption spectrum showed
 a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.23.

15

EXAMPLE 63

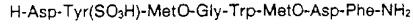


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The title compound has previously been prepared (U.S. Patent 3,892,726). H-DPhe-OCH₂-Pam-resin
 {prepared in an analogous fashion as H-Phe-OCH₂-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-
 acetic acid replaced with Boc-DPhe-(4-oxymethylphenyl)acetic acid] from Boc-DPhe-(4-oxymethylphenyl)-
 25 acetic acid [Example 5, Boc-MePhe-OH replaced with Boc-DPhe-OH]} was sequentially coupled with Fmoc-
 Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3),
 and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to
 provide Boc-Asp (OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-DPhe-OCH₂-Pam-resin which was sulfated, de-
 protected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-
 30 29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE
 according to Table 1 (Step 30) to give 141 mg of the ammonium salt of the title compound. Amino acid
 analysis following acid decomposition gave Asp 1.97 (2), Tyr 0.98 (1), Met 2.03 (2), Gly 1.09 (1), and Phe
 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹.
 TLC R_f 0.32.

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EXAMPLE 64



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The title compound has previously been prepared (Nobel Symp., 16, (Front. Gastrointest. Horm. Res.),
 41-56 (1973)). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH,
 Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-MetO-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp-
 (OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide
 45 Boc-Asp(OtBu)-Tyr-MetO-Gly-Trp-MetO-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected,
 and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with
 ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE
 according to Table 1 (Step 30) to give 162 mg of the ammonium salt of the title compound. Amino acid
 analysis following acid decomposition gave Asp 2.09 (2), Tyr 1.00 (1), MetO 1.83 (2), Gly 1.08 (1), and Phe
 50 1.08 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹.
 TLC R_f 0.14.

EXAMPLE 65

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H-Asp-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂

The title compound has previously been prepared (U.S. Patent 3,892,726). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Bos-Asp(OtBu)-Tyr-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 94 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.18 (2), Tyr 1.06 (1), Ahx 2.01 (2), Gly 1.10 (1), and Phe 0.94 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.32.

EXAMPLE 66

H-Asp-Tyr(SO₃H)-Leu-Gly-Trp-Leu-Asp-Phe-NH₂

The title compound has previously been prepared (Digestive Diseases, 15, 149-156 (1970)). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Leu-Gly-Trp-Leu-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 150 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 0.99 (1), Leu 2.02 (2), Gly 0.98 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.35.

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EXAMPLE 67

For-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

The title compound has previously been prepared (U.S. Patent 3,705,140). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Met-Gly-Trp-Met-Asp-Phe-OCH₂-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (Steps 3-4) to give For-Tyr-Met-Gly-Trp-Met-Asp-Phe-OCH₂-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.00 (1), Met 1.98 (2), Gly 1.07 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

EXAMPLE 68

H-Asp-Tyr(SO₃H)-MetO-Gly-Trp-Met-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OTBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-MetO-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-MetO-Gly-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 94 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.18 (2), Tyr 1.06 (1), Met 1.98 (2), Gly 1.07 (1), and Phe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.32.

ed from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 230 mg of the ammonium salt of the title compound. Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.26.

5

EXAMPLE 69

H-Asp-Tyr(SO₃H)-Met-DAla-Trp-Met-Asp-Phe-NH₂

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The title compound has previously been prepared (Peptides 1984, 383-385 (1984)). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-DAla-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and Amberlite XAD-2, sequentially, according to Table 1 (Step 30) to give 140 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.93 (2), Tyr 0.94 (1), Met 1.81 (2), Ala 0.97 (1), and Phe 0.95 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.34.

EXAMPLE 70

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H-Asp-Tyr(SO₃H)-Met-Gly-Trp-DMet-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-DMet-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-Trp-DMet-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 368 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.90 (2), Tyr 0.99 (1), Met 1.97 (2), Gly 0.92 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.31.

EXAMPLE 71

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H-Asp-Tyr(SO₃H)-Met-Gly-Trp-MetO-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-MetO-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-Trp-MetO-Asp(OtBu)-Phe-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE according to Table 1 (Step 30) to give 126 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.03 (2), Tyr 0.96 (1), Met 0.98 (1), Gly 1.10 (1), MetO 0.97 (1), and Phe 0.96 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.29.

55 EXAMPLE 72

H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe(4-Me)-NH₂

H-Phe(4-Me)-OCH₂-Pam-resin {prepared in an analogous fashion as H-Phe-OCH₂-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-acetic acid replaced with Boc-Phe(4-Me)-(4-oxymethylphenyl)acetic acid] from Boc-Phe(4-Me)-(4-oxymethylphenyl) acetic acid [Example 5, Boc-MePhe-OH replaced with Boc-Phe(4-Me)-OH]} was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-Phe(4-Me)-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 420 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.02 (2), Tyr 1.00 (1), Met 1.91 (2), Gly 1.06 (1), and Phe (4-Me) 1.12 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.44.

EXAMPLE 73

20 H-Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Tyr-NH₂

H-Tyr(tBu)-OCH₂-Pam-resin {prepared in an analogous fashion as H-Phe-OCH₂-Pam-resin [Example 11, Boc-Phe-(4-oxymethylphenyl)-acetic acid replaced with Fmoc-Tyr(tBu)-(4-oxymethylphenyl)-acetic acid] from Boc-Tyr(tBu)-(4-oxymethylphenyl) acetic acid [Example 5, Boc-MePhe-OH replaced with Fmoc-Tyr(tBu)-OH]} was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Met-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Tyr-OH (Example 3), and Boc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Boc-Asp(OtBu)-Tyr-Met-Gly-Trp-Met-Asp(OtBu)-Tyr(tBu)-OCH₂-Pam-resin which was sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 21-25, Steps 10-15, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 42 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.09 (2), Tyr 2.16 (2), Met 1.66 (2), and Gly 1.09 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.29.

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EXAMPLE 74

40 Hpp(SO₃H)-Met-Gly-Trp-Pro-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Met-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Met-Gly-Trp-Pro-Asp-(OtBu)-Phe-OCH₂-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Met-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 70 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Met 1.01 (1), Gly 1.04 (1), Pro 0.97 (1), Asp (1.04) (1), and Phe 0.94 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.51.

EXAMPLE 75

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Hpp(SO₃H)-Pro-Gly-Trp-Pro-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Pro-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Pro-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Pro-Gly-Trp-Pro-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 480 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Pro 2.02 (2), Gly 1.05 (1), Asp 1.00 (1), and Phe 0.93 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.44.

EXAMPLE 76**Suc-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂**

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2, Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 100 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.97 (1), Gly 1.00 (1), and Asp 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.46.

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EXAMPLE 77**iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂**

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 290 mg of the ammonium salt of the title compound. Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.54.

EXAMPLE 78**Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-MePhe-NH₂**

H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ahx-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ahx-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ahx-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Steps 8-9) to give Hpp-Ahx-Gly-Trp-Ahx-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2,

Trisacryl M DEAE, and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 91 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ahx 1.92 (2), Gly 1.03 (1), and Asp 1.05 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.40.

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EXAMPLE 79

iBuOCO-Tyr(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂

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H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ahx-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 800 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.90 (1), Ahx 1.84 (2), Gly 1.12(1), Asp 1.12 (1), and Phe 0.92 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.58.

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EXAMPLE 80

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Hpp(SO₃H)-Ahx-Gly-Trp-Ahx-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Asp(OtBu)-OH, Fmoc-Ahx-OH, Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ahx-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Step 8-9) to give Hpp-Ahx-Gly-Trp-Ahx-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 450 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ahx 1.92 (2), Gly 1.01(1), Asp 1.10(1), and Phe 0.97(1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.47.

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EXAMPLE 81

Suc-Tyr(SO₃H)-Met-DAla-Trp-Met-Asp-Phe-NH₂

The title compound has previously been prepared (Peptides 1984, 373-378 (1984)). H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10), Fmoc-Trp-OH, Fmoc-DAla-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 3-4 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Met-DAla-Trp-Met-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Trisacryl M DEAE and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 110 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.02 (1), Met 1.93 (2), Ala 1.00 (1), Asp 1.05 (1), and Phe 1.01 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.38.

EXAMPLE 82

For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Ile-Gly-Trp-Ile-Asp-Phe-OCH₂-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (Steps 3-4) to give For-Tyr-Ile-Gly-Trp-Ile-Asp-Phe-OCH₂-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 200 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.98 (1), Ile 1.94 (2), Gly 1.00 (1), Asp 1.09 (1), and Phe 0.98 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.59.

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EXAMPLE 83

Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr-(Bu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(Bu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(Bu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 300 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.00 (1), Ile 2.07 (2), Gly 0.97 (1), Asp 0.97 (1), and Phe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.42.

EXAMPLE 84

iBuOCO-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr-(Bu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(Bu)-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(Bu)-Ile-Gly-Trp-Ile-Asp(OtBu)-PheOCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 390 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.07 (1), Ile 2.21 (2), Gly 1.04 (1), Asp 1.00 (1), and Phe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.61.

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EXAMPLE 86

Hpp (SO₃H)-Ile-Gly-Trp-Ile-Asp-Phe-NH₂

H-Phe-OCH₂-Pam-resin (Example 11) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was coupled with Hpp-OSu in DMF according to Table 1 (Step 8-9) to give Hpp-Ile-Gly-Trp-Ile-Asp(OtBu)-Phe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the

resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 280 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ile 2.04 (2), Gly 0.99 (1), Asp 0.98 (1), and Phe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.52.

EXAMPLE 87

10 Suc-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-MePhe-NH₂

The title compound has previously been prepared (N. Yanaihara, et al., in Peptides 1984: Proceedings of the 18th European Peptide Symposium, U. Ragnarsson, Ed., Almqvist and Wiksell International, Publisher, Stockholm, Sweden, 1985, pp 373-378). H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Met-Asp(OtBu)-OH (Example 10) Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 170 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.02 (1), Met 1.94 (2), Gly 1.09 (1), Asp 1.00 (1), and MePhe 1.02 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.58.

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EXAMPLE 88

30 H-DAsp-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂

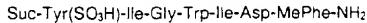
H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-DAsp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-DAsp(OtBu)-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 30 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 1.96 (2), Tyr 0.97 (1), Ile 1.92 (2), Gly 0.98 (1), and MePhe 1.18 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.36.

EXAMPLE 89

45 For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂

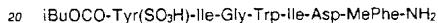
H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected according to Table 1 (Steps 10-15) to provide H-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin which was coupled with For-Tyr-OH according to Table 1 (coupling Steps 3-4) to give For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin which was sulfated and cleaved from the resin according to Table 1 (Steps 21-25 and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 LDS-3, sequentially, according to Table 1 (Step 30) to give 90 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.04 (1), Ile 1.95 (2), Gly 0.97 (1), Asp 1.01 (1), and MePhe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.60.

EXAMPLE 90



5 H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with succinic anhydride in DMF according to Table 1 (Steps 8-9) to give Suc-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 130 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 0.94 (1), Ile 1.92 (2), Gly 1.04 (1), Asp 1.09 (1), and MePhe 1.00 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.37.

EXAMPLE 91



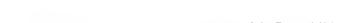
25 H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, and Fmoc-Tyr(tBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with iBuOCO-OSu (Example 1) in DMF according to Table 1 (Steps 8-9) to give iBuOCO-Tyr(tBu)-Ile-Gly-Trp-Ile-Asp-(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 50 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Tyr 1.00 (1), Ile 1.92 (2), Gly 1.02 (1), Asp 1.03 (1), and MePhe 1.03 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.74.

35 EXAMPLE 92



40 H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-DTyr(tBu)-OH, and Fmoc-Asp(OtBu)-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide Fmoc-Asp(OtBu)-DTyr(tBu)-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-Resin which was deprotected, sulfated, deprotected, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, Steps 16-20, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 200 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Asp 2.08 (2), Tyr 1.01 (1), Ile 1.94 (2), Gly 0.98 (1), and MePhe 0.99 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.38.

50 EXAMPLE 93

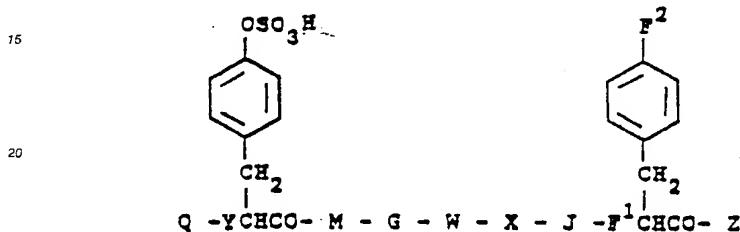


55 H-MePhe-OCH₂-Pam-resin (Example 12) was sequentially coupled with Fmoc-Ile-Asp(OtBu)-OH (Example 10, Fmoc-Met-OH replaced with Fmoc-Ile-OH), Fmoc-Trp-OH, Fmoc-Gly-OH and Fmoc-Ile-OH according to Table 1 (coupling Steps 5-7 followed by Fmoc removal Steps 16-20) to provide H-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was coupled with Hpp-OSu according to Table 1 (coupling

Steps 8-9) to give Hpp-Ile-Gly-Trp-Ile-Asp(OtBu)-MePhe-OCH₂-Pam-resin which was deprotected, sulfated, and cleaved from the resin according to Table 1 (Steps 10-15, Steps 21-25, and then Steps 26-29 with ammonia) to give the title compound which was chromatographically purified on Amberlite XAD-2 and P-40 ODS-3, sequentially, according to Table 1 (Step 30) to give 70 mg of the ammonium salt of the title compound. Amino acid analysis following acid decomposition gave Ile 1.94 (2), Gly 1.01 (1), Asp 1.02 (1), and MePhe 1.04 (1). Infrared absorption spectrum showed a strong peak typical of a sulfuric acid ester at 1050 cm⁻¹. TLC R_f 0.63.

10 Claims

1. A peptide of the formula



25 wherein

Q is H-βAsp, For, Suc, desQ, or R¹R²CHOCO,

Y is H or (S)-NH,

M is Met, Ahx, Leu, or Ile,

G is Gly,

30 W is Trp,

X is Met, Ahx, Leu, or Ile,

J is Asp,

F¹ is (S)-NH or (S)-R⁴N,35 F² is H, NO₂, R⁵, or OR⁶,Z is NH₂R¹ and R² are independently H or lower alkyl,R³, R⁴, R⁵, and R⁶ are lower alkyl,

and pharmaceutically acceptable salts thereof,

provided that

(1) Q is desQ when Y is H,

(2) F² is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F¹ is (S)-NH,(3) F² is not H if, in the same peptide, Y is H, M is Met, X is Met, and F¹ is (S)-NH.45 (4) F² is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F¹ is (S)-R⁴N.

2. A peptide of Claim 1 in which Q is iBuOCO.

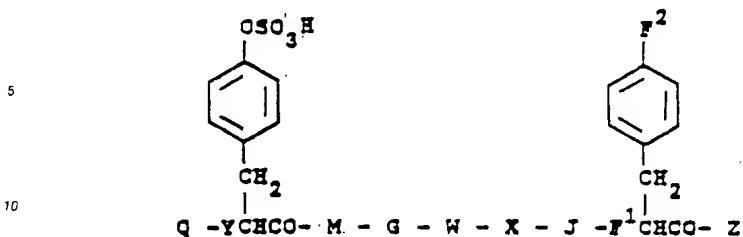
3. A peptide of Claim 1 in which Q is Suc.

4. A peptide of Claim 1 in which Q is For.

5. A peptide of the formula

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wherein

Q is H, H-Asp-H- β Asp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R¹R²CHOCO,

Y is (R)-NH,

M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, Melle or Lys,

G is Gly, DAla, Pro or Sar,

W is Trp, MeTrp or Nal,

X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, Melle, Pro, or Lys,

J is Asp, DAsp, MeAsp, or Asn,

20

F¹ is (S)-NH, (S)-R⁴N, or (R)-R⁴N,

F² is H, Cl, I, Br, F, NO₂, NH₂, R⁵, or OR⁶,

Z is NH₂, NHR⁷ or NR⁷R⁸,

R¹ and R² are independently H or lower alkyl,

25

R³, R⁴, and R⁵ are lower alkyl,

R⁶ is H or lower alkyl, and

R⁷ and R⁸ are lower alkyl,

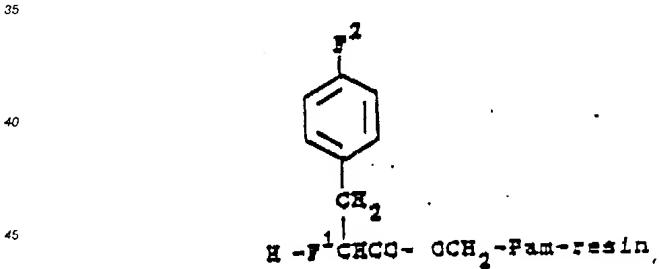
and pharmaceutically acceptable salts thereof,

provided that

30

(1) F² is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F¹ is (S)-NH and Z is NH₂.

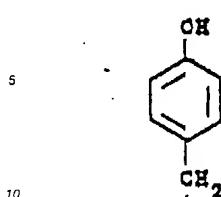
6. A process for preparing a compound of Claim 1 or 5 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl resin of the formula



to afford a protected peptidyl resin of the formula

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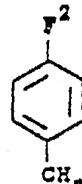
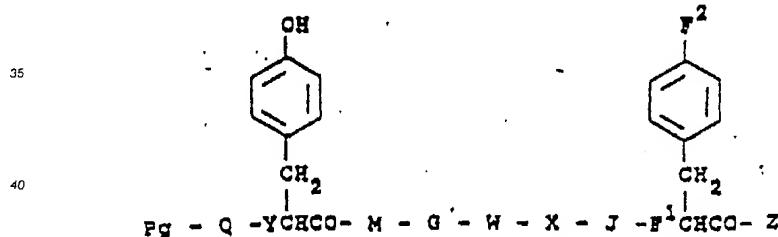


which is sulfated, deprotected, and treated with a methanolic amine.

15 7. The process for preparing a compound of Claim 1 or 5 by the solution phase method of sequential addition (followed by deprotection after each addition) to the requisite protected amino acid or protected oligo peptide to an amino-acyl amide of the formula



30 to afford a protected peptide amide of the formula



which is sulfated and deprotected.

45 8. The peptide H-DAsp-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂.
 9. The peptide For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂.
 10. The peptide Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂.
 11. The peptide iBuOCC-Tyr(SO₃H)-Ile-Gly-Trp-Asp-MePhe-NH₂.
 12. The peptide Hpp(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂.
 13. The peptide of claim 5 that is H-Asp-DTyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂.
 14. A process for preparing the compound of claim 8 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin H-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.
 15. A process for preparing the compound of claim 9 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

16. A process for preparing the compound of claim 10 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin Suc-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

17. A process for preparing the compound of claim 11 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin iBuOCO-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

18. A process for preparing the compound of claim 12 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin Hpp-Ille-Gly-Trp-Ille-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

19. A process for preparing the compound of claim 13 by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin H-Asp-DTyr-Ille-Gly-Trp-Ille-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

20. A process for preparing the compound of claim 8 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-DAsp-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-NH₂, which is sulfated and deprotected.

21. A process for preparing the compound of claim 9 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-For-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-NH₂, which is sulfated and deprotected.

22. A process for preparing the compound of claim 10 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Suc-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-NH₂, which is sulfated and deprotected.

23. A process for preparing the compound of claim 11 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-iBuOCO-Tyr-Ille-Gly-Trp-Ille-Asp-MePhe-NH₂, which is sulfated and deprotected.

24. A process for preparing the compound of claim 12 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Hpp-Ille-Gly-Trp-Ille-Asp-MePhe-OCH₂-NH₂, which is sulfated and deprotected.

25. A process for preparing the compound of claim 13 by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Asp-DTyr-Ille-Gly-Trp-Ille-Asp-MePhe-NH₂, which is sulfated and deprotected.

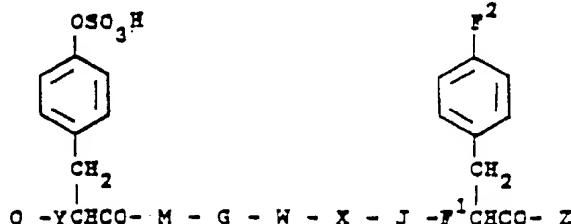
Claims for the following contracting States: GR and ES.

45 1. A process for preparing a peptide of the formula

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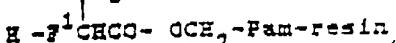
wherein

Q is H- β Asp, For, Suc, desQ, or R¹R²CHOCO,
 Y is H or (S)-NH,
 M is Met, Ahx, Leu, or Ile,
 G is Gly,
 5 W is Trp,
 X is Met, Ahx, Leu, or Ile,
 J is Asp,
 F¹ is (S)-NH or (S)-R⁴N,
 F² is H, NO₂, R⁵, or OR⁶,
 10 Z is NH₂
 R¹ and R² are independently H or lower alkyl,
 R³, R⁴, R⁵, and R⁶ are lower alkyl,
 and pharmaceutically acceptable salts thereof,
 provided that
 15 (1) Q is desQ when Y is H,
 (2) F² is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X
 is either Met, Ahx, or Leu, and F¹ is (S)-NH,
 (3) F² is not H if, in the same peptide, Y is H, M is Met, X is Met, and F¹ is (S)-NH,
 (4) F² is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F¹ is (S)-R⁴N, by the
 20 solid phase method of sequential addition (followed by deprotection after each addition) of the requisite
 protected amino acid or protected oligo peptide to an amino-acyl resin of the formula

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to afford a protected peptidyl resin of the formula

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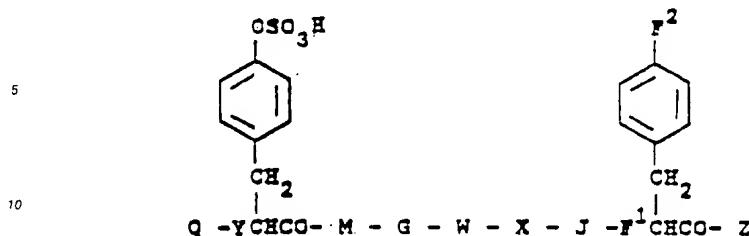


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which is sulfated, deprotected, and treated with a methanolic amine.

2. A process for preparing a peptide of the formula

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wherein

Q is H, H-Asp, H- β Asp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R¹R²CHOCO,

Y is (R)-NH₂

15 N is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, Melle or Lys.

G is Gly, DAla, Pro or Sar

W is Trp, MeTrp or Nal

Y is Met, MeMet, MetO

X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MeIle, Pro, or Lys, His, Arg, DArg, MeArg, or Aib.

20 J is Asp, UAsp, MeAsp, or Asn.

F¹ is (S)-NH, (S)-R⁴N, or (R)-R⁴N.

F^2 is H, Cl, I, Br, F, NO_2 , NH_2 , R^3 , or OR^6 .

Z is NH_2 , NHR^7 or NR^7R^8 ,

R¹ and R² are independently H or lower alkyl.

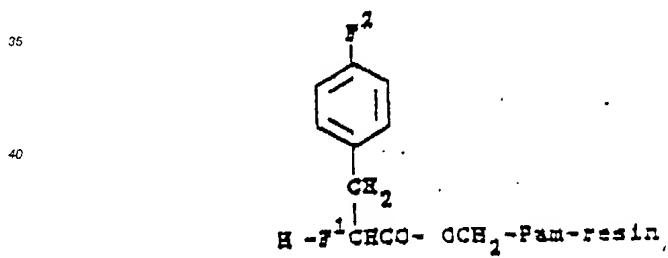
R^3 , R^4 , and R^5 are lower alkyl.

R⁶ is H or lower alkyl, and

R⁷ and R⁸ are lower alkyl.

and pharmaceutically acceptable salts thereof,

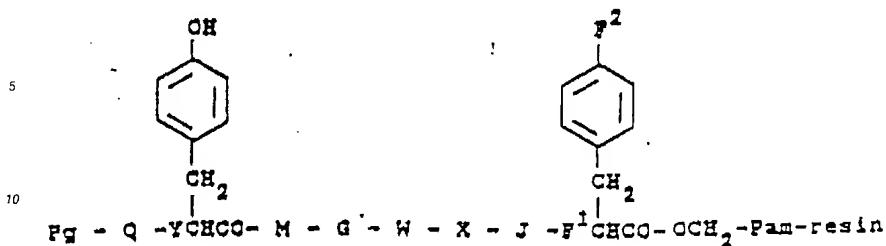
30 (1) F² is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F¹ is (S)-NH and Z is NH₂, by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to an amino-acyl resin of the formula:



to afford a protected peptidyl resin of the formula

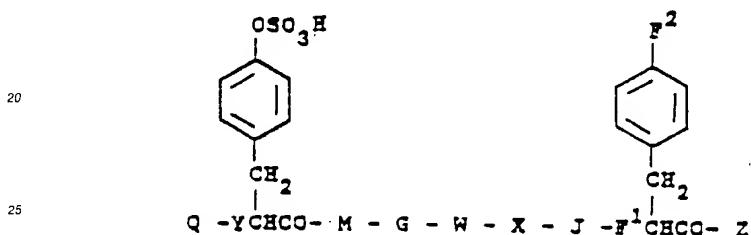
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which is sulfated, deprotected, and treated with a methanolic amine.

16 3. A process for preparing a peptide of the formula



wherein

Q is H- β Asp, For, Suc, desQ, or R¹R²CHOCO,

30 Y is H or (S)-NH,

M is Met, Ahx, Leu, or Ile,

G is Gly,

W is Trp,

X is Met, Ahx, Leu, or Ile,

35 J is Asp,

F¹ is (S)-NH or (S)-R⁴N,

F² is H, NO₂, R⁵, or OR⁶,

Z is NH₂,

40 R¹ and R² are independently H or lower alkyl,

R³, R⁴, R⁵, and R⁶ are lower alkyl,

and pharmaceutically acceptable salts thereof,

provided that

(1) Q is desQ when Y is H,

(2) F² is not H if, in the same peptide, Q is H-BAsp, For, or Ac, Y is (S)-NH, M is either Met, Ahx, or Leu, X is either Met, Ahx, or Leu, and F¹ is (S)-NH,

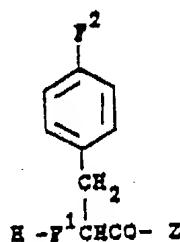
(3) F² is not H if, in the same peptide, Y is H, M is Met, X is Met, and F¹ is (S)-NH,

(4) F² is not H if, in the same peptide, Q is Suc, Y is (S)-NH, M is Met, X is Met, and F¹ is (S)-R⁴N, by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligo peptide to an amino-acyl amide of the formula

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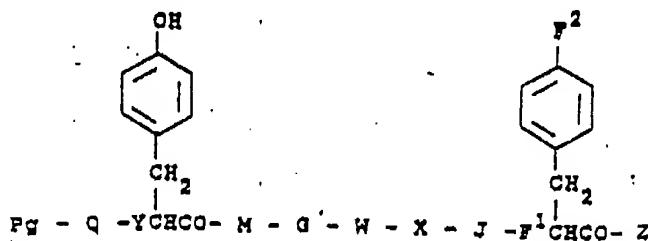


to afford a protected peptide amide of the formula

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which is sulfated and deprotected.

4. A process for preparing a peptide of the formula

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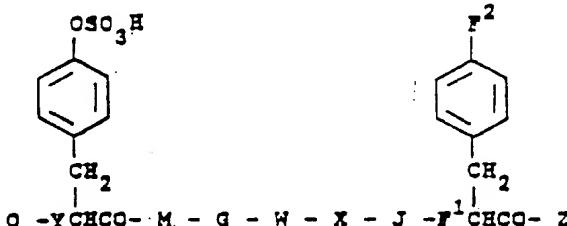
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wherein

Q is H, H-Asp, H- β -Asp, H-DAsp, H-MeAsp, For, Ac, Suc, desQ, or R¹R²CHOCO,

Y is (R)-NH,

M is Met, DMet, MeMet, MetO, Ahx, DAhx, MeAhx, Leu, MeLeu, Pro, Ile, MetIle or Lys.

G is Gly, DAla, Pro or Sar,

W is Trp, MeTrp or Nal.

X is Met, MeMet, MetO, Ahx, MeAhx, Leu, MeLeu, Ile, MetIle, Pro, or Lys.

J is Asp, DAsp, MeAsp, or Asn,

F¹ is (S)-NH, (S)-R⁴N, or (R)-R⁴N,

F² is H, Cl, I, Br, F, NO₂, NH₂, R⁵, or OR⁶,

Z is NH₂, NHR⁷ or NR⁷R⁸,

R¹ and R² are independently H or lower alkyl,

R³, R⁴, and R⁵ are lower alkyl,

R⁶ is H or lower alkyl, and

R⁷ and R⁸ are lower alkyl,

and pharmaceutically acceptable salts thereof,

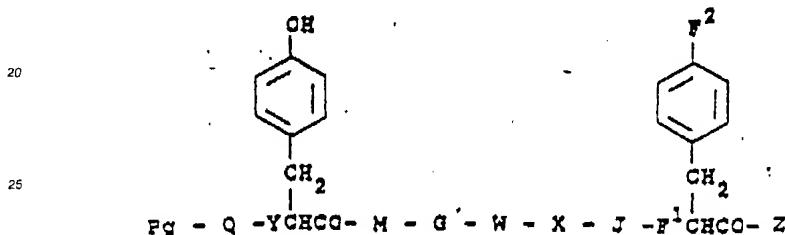
provided that

(1) F² is not H if, in the same peptide, Q is H-Asp, M and X are Ile, G is Gly, J is Asp, F¹ is (S)-NH and Z is

NH₂, by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to an amino-acyl amide of the formula



15 to afford a protected peptide amide of the formula



which is sulfated and deprotected.

30 5. A process for preparing the compound H-DAsp-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin H-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

35 6. A process for preparing the compound For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

40 7. A process for preparing the compound Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

45 8. A process for preparing the compound iBuOCO-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

50 9. A process for preparing the compound Hpp(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanolic amine.

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10. A process for preparing the compound H-Asp-DTyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solid phase method of sequential addition (followed by deprotection after each addition) of the requisite protection amino acid or protected oligopeptide to the amino-acyl resin H-MePhe-OCH₂-Pam-resin to afford the peptidyl resin H-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-Pam-resin, which is sulfated and treated with a methanoic amine.

5 11. A process for preparing the compound H-DAsp-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-DAsp-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, which is sulfated and deprotected.

10 12. A process for preparing the compound For-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-For-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, which is sulfated and deprotected.

15 13. A process for preparing the compound Suc-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Suc-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, which is sulfated and deprotected.

20 14. A process for preparing the compound iBuOCO-Tyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-iBuOCO-Tyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, which is sulfated and deprotected.

25 15. A process for preparing the compound Hpp(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Hpp-Ile-Gly-Trp-Ile-Asp-MePhe-OCH₂-NH₂, which is sulfated and deprotected.

30 16. A process for preparing the compound H-Asp-DTyr(SO₃H)-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂ by the solution phase method of sequential addition (followed by deprotection after each addition) of the requisite protected amino acid or protected oligopeptide to H-MePhe-NH₂ to afford the protected peptide amide Pg-Asp-DTyr-Ile-Gly-Trp-Ile-Asp-MePhe-NH₂, which is sulfated and deprotected.

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EUROPEAN PATENT APPLICATION

(2) Application number: 87117096.5

(5) Int. Cl.⁵ C07K 7/30

(3) Date of filing: 19.11.87

(19) Priority: 18.11.86 US 932119
27.05.87 US 54554

(7) Applicant: FISONS CORPORATION
Jefferson Road
Rochester, NY 14603(US)

(43) Date of publication of application:
25.05.88 Bulletin 88/21

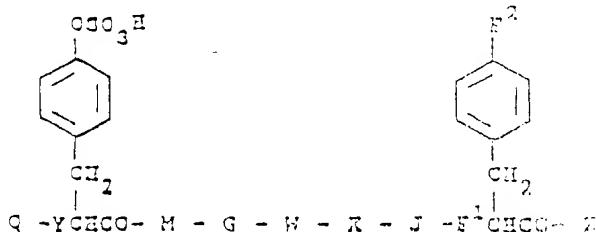
(2) Inventor: Rosamond, James D.
187 Burlington Avenue
Rochester N.Y. 14619(US)

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(74) Representative: Wright, Robert Gordon McRae
FISONS plc 12 Derby Road
Loughborough Leicestershire LE11 0BB(GB)

(2) Peptides with sulfate ester groups.

(57) Novel peptides having sulfate ester groups and containing 6 to 9 amino acids of the formula



possess feeding inhibition properties and capable of stimulating the contraction of the gallbladder. Also methods of treating and preventing obesity in which these novel peptides or other specified peptides can be used.

EP 0 268 297 A3



European Patent
Office

EUROPEAN SEARCH REPORT

Application number
EP 87 11 7096

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl 4)
X	CHEMICAL ABSTRACTS, vol. 104, no. 21, May 26, 1986, page 511, abstract no. 184817b, Columbus, Ohio, US; & JP-A-60 197 699 (FUJI CHEMICALS INDUSTRIAL CO. LTD.) 07-10-1985 * Abstract * --		C 07 K 7/30
Y	CHEMICAL ABSTRACTS, vol. 102, no. 15, April 15, 1985, pages 671, abstract no. 132473r, Columbus, Ohio, US; & EP-A-0 124 420 (SANOFI; CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE) 07-11-1984 * Abstract * --	1-5, 7	
Y	CHEMICAL ABSTRACTS, vol. 89, no. 13, September 25, 1978, page 1006, abstract no. 110419p, Columbus, Ohio, US; & DD-A-2 751 026 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 01-06-1978 * Abstract * --	1-7, 9-12, 15-18, 21-24	TECHNICAL FIELDS SEARCHED (Int. Cl 4) C 07 K
		1-7, 9-12, 15-18, 21-24	
		. / .	
TRANSLATION OF DOCUMENTS COMPLETED IN THIS REPORT			
Place of search	Date of completion of the search	Examiner	
THE HAGUE	12-02-1990	MASTURZO	
CATEGORY OF CITED DOCUMENTS			
X	particularly relevant if taken alone	T	: theory or principle underlying the invention
Y	particularly relevant if combined with another document of the same category	E	: earlier patent document, but published on, or after the filing date
A	technological background	D	: document cited in the application
O	non-written disclosure	L	: document cited for other reasons
P	intermediate document	&	: member of the same patent family, corresponding document



European Patent
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CLAIMS INCURRING FEES

The present European patent application comprises at the time of filing more than ten claims.

- All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.
namely claims:
- No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X | LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

- All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.
namely claims:
- None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.
namely claims: 1-7 (partially), 9-12, 15-18, 21-24 (completely)



European Patent
Office

EUROPEAN SEARCH REPORT

Application number

EP 87 11 7096

-2-

DOCUMENTS CONSIDERED TO BE RELEVANT

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (int Cl +)
Y	CHEMICAL ABSTRACTS, vol. 97, no. 25, December 20, 1982, page 905, abstract no. 216686d, Columbus, Ohio, US; M. BODANSZKY et al.: "Application of the 9-fluorenylmethoxy-carbonyl (FMOC) group in peptide synthesis in solution" & PEPT., PROC. EUR. PEPT. SYMP., 16th 1980 (Pub. 1981), 93-8 * Abstract *	1-7, 9-12, 15-18, 21-24	
Y	US-A-4 490 364 (RIVIER et al.) * The whole document *	1-7, 9-12, 15-18, 21-24	<small>TECHNICAL FIELDS SEARCHED (int Cl +)</small>
Y	US-A-4 351 829 (ZETLER et al.) * The whole document, especially column 1, lines 1-20 *	1-7, 9-12, 15-18, 21-24	
Y	EP-A-0 107 860 (AMANO) * The whole document, especially page 46, claim 1 *	1-7, 9-12, 15-18, 21-24	
./.			
X54 X000K34K56X000XH X000G000X000XH X000X000X			
Place of search		Date of completion of the search	Examiner
CATEGORY OF CITED DOCUMENTS			
X	particularly relevant if taken alone	T	theory or principle underlying the invention
Y	particularly relevant if combined with another document of the same category	E	earlier patent document, but published on, or after the filing date
A	technological background	D	document cited in the application
O	non-written disclosure	L	document cited for other reasons
P	intermediate document	&	member of the same patent family, corresponding document

CATEGORY OF CITED DOCUMENTS

T. theory or principle underlying the invention

E : earlier patent docu

after the filing date

document cited in the application
 document cited for other reasons

E : document cited for other reasons

& member of the same patent family, corresponding document



European Patent
Office

EUROPEAN SEARCH REPORT

Application number

EP 87 11 7096

- 3 -

DOCUMENTS CONSIDERED TO BE RELEVANT

P07 Envir 1403 0302